PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 12316450/EJH	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/AU2003/001040**	International Filing Date *(day/month/year)* 15 August 2003	Priority Date *(day/month/year)* 15 August 2002

International Patent Classification (IPC) or national classification and IPC

Int. Cl.[7] G01N 33/536, 15/10.

Applicant

THE CORPORATION OF THE TRUSTEES OF THE ORDER OF THE SISTERS OF MERCY IN QUEENSLAND *et al.*

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☒	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
12 February 2004	29 November 2004
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **NORMAN BLOM** Telephone No. (02) 6283 2238

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/001040

I. Basis of the report

1. With regard to the elements of the international application:*

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[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , received on with the letter of

[ ] the drawings, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	**none**	**YES**
	Claims	**1-29**	**NO**
Inventive step (IS)	Claims	**none**	**YES**
	Claims	**1-29**	**NO**
Industrial applicability (IA)	Claims	**1-29**	**YES**
	Claims	**none**	**NO**

2. Citations and explanations (Rule 70.7)

Reference is made to the following documents

D1: **Proc. Natl. Acad. Sci. USA**, (1997), **94**, 12551-12556,

D2: **The Journal of Immunology** (1999), 3250-3259,

D3: **Brain** (2001), **124**, 480-492,

D4: **Proc. Natl. Acad. Sci. USA**, (1995), **92**, 826-830,

D5: **The Journal of Immunology**, (2000),**165** (11), 6037-6046,

D6: **Cancer Immunol Immunother**. (1998), **45**, 234-240,

D7: **Cytometry** (2000), **40**, 50-59,

D8: **American Journal of Pathology** (2001), **159** (1), 285-295,

D9: BD Biosciences, Reagents Dendritic Cell Identification, Application Note "Peripheral Blood Dendritic Cells Revealed by Flow Cytometry, Identification of CD123+ (anti-interleukin 3 receptor α chain) and CD11c+ dendritic cell subsets", © 2000 Becton, Dickinson and Company (www:bdbiosciences.com/immunocytometry_systems/application_notes/pdf/reagapp3.pdf),

D10: **Clinical Cancer Research** (June 2002), **8**, 1787-1793,

D11: **WO 2001/027245**.

Novelty (N) and Inventive Step (IS): Claims 1-29

D1 discloses that tonsils contain dendritic cells (DCs) that can be identified as cells that lack lineage markers for monocytes and lymphocytes (lin^-) (CD3, CD14, CD16, CD19, CD20, CD56 and goat-anti-human IgM) and are positive for HLA-DR and CD4 ($HLA\text{-}DR^+CD4^+lin^-$). 85% of these cells react with antibodies to IL-3Rα. Tonsillar and PBMC $HLA\text{-}DR^+lin^-IL\text{-}3R\alpha^{hi}$ cells were sorted by FACS and were readily detected as a population with low orthogonal light scatter in blood. Table 1 lists the presence or absence of 28 surface molecules on $HLA\text{-}DR^+lin^-IL\text{-}3R\alpha^{hi}$ DC from tonsils, these cells are $CD3^-$, $CD11b^-$, $CD11c^-$, $CD14^-$, $CD16^-$, $CD19^-$, $CD34^{+/-}$, $CD40^{++}$, $CD56^-$, $HLA\text{-}DR^{++}$. Claims 1-2, 4-9, 11, 16, 19, 22-26, at least, are considered to lack novelty and an inventive step in the light of this disclosure.

(continued)

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date (valid claim) *(day/month/year)*
P,X WO 2003/005972	23 January 2003	15 July 2002	13 July 2001

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 1 is not clear because the method is defined as placing **one** or more immunointeractive molecules in contact with a sample wherein said immunointeractive molecules are directed against **one** or more dendritic cell immunogens **and one** or more non-dendritic cell immunogens. Clearly the method involves the use of **two** or more immunointeractive molecules.

Claims 2 and 3 are not clear with regard to the meaning of "subclasses" as used in step (d) "optional steps of isolating cells based in part on the presence of dendritic cell immunogens from previous **subclasses** (a,b,c)".

Claim 16 is not clear as numerous of the citations list CD16 as a non-dendritic cell immunogen whereas in this claim it is suggested to be a dendritic cell immunogen (see for example documents D1-D4, D6-D10 and D12).

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of V. 2. Citations and explanations (Rule 70.7)

D2 discloses the isolation of peripheral blood dendritic cells using Ficoll Hypaque gradient centrifugation, counter current elutriation and immunomagnetic depletion (MACS depletion) of remaining $CD3^+$, $CD11b^+$, $CD16^+$, $CD19^+$, $CD34^+$, $CD56^+$ cells (non DC). The DC enrichment in individual elutriation fractions was monitored by analysing the frequency of $HLA\text{-}DR^+CD4^+lin^-$ PBMC and/or the cellular light scatter profiles using a FACScan. Blood and CSF mononuclear cells were stained with FITC-labelled anti-CD3, CD14, CD16, CD19, and CD56, PE-labelled anti-CD123 and PerCP-labelled anti-HLA-DR and analysed by a three colour FACScan flow cytometer. Mononuclear cells were distinguished from debris by forward/sideward light scattering (the dendritic cells are larger in size (higher forward scatter) and have slightly higher granularity (higher side scatter) than non-dendritic cells, which were mainly T cells). Plasmacytoid CSF DCs were isolated by means of a FACSVantage cell sorter. Claims 1-2, 4-11, 16, 18, 22-28 are considered to lack novelty and an inventive step in the light of this disclosure.

D3 discloses the flow cytometric identification of dendritic cell in blood and CSF based on their lack of markers for T cell, B cells, natural killer cells or monocytes, positivity for HLA-DR and expression of CD123 (for plasmacytoid dendritic cells) and CD11c (for myeloid dendritic cells). Table 2 list other monoclonal antibodies used in the study, including CD3, CD11c, CD14, CD16, CD19, CD40, CD56, CD83, CD123 and HLA-DR. It is indicated that DC are larger in size (higher forward scatter) and have slightly higher granularity (higher side scatter) than non-dendritic cells (see Fig. 2). Claims 1-2, 4-11, 16, 22-26, at least, are considered to lack novelty and an inventive step in the light of this disclosure.

D4 discloses the identification of three populations of cells in peripheral blood that have or can develop a dendritic morphology, only one of which is infectable with HIV type 1. Also disclosed is a three-colour cytofluorometric analysis of freshly isolated PBMCs, without *in vitro* culture, by staining with (1) HB15 and fluorescein-labelled goat anti mouse $F(ab')_2$; (2) phycoerythrin-labelled anti-CD3, -CD14, -CD16, -CD19, -CD20, and -CD56 (lineage markers); and (3) red670 conjugated anti-HLA-DR and analysed on a Coulter EPICS C flow cytometer. The cells that did not stain with a mixture of mAbs to T, B, NK and monocytic cells were analysed for expression of HLA-DR and HB15. A population of $HLA\text{-}DR^{bright}$ and $HB15^+$ accounted for 0.05-0.1% of the total PBMCs. Claims 1-2, 4-9, 22-25, at least, are considered to lack novelty and an inventive step in the light of this disclosure.

D5 discloses three new markers, BDCA-2, BDCA-3 and BDCA-4, for distinct subsets of DCs in human peripheral blood. The purity of BDC was demonstrated by light scatter properties and anti-HLA-DR-Cy5 verse anti lin-FITC (anti-TCRαβ, CD14, CD19, and CD56). BDCA-2 and BDCA-4 are expressed on $CD11c^-$ $CD123^{bright}$ plasmacytoid DCs whereas BDCA-3 is expressed on a small population of $CD11c^+$ $CD123^-$ DCs. Table II lists the surface phenotype of $BDCA\text{-}2^+$ BDCs, $BDCA\text{-}3^+$ BDCs and $CD1c^+$ BDCs (including CD1c, CD11c, CD16, CD40, CD83, CD123, HLA-DR, CMRF44, CMRF56). Claims 1-2, 4-11, 13-17, 19-21, 24-26, at least, are considered to lack novelty and an inventive step in the light of this disclosure.

D6 discloses an investigation into the feasibility of using multidimensional flow cytometry to analyze DC directly in peripheral blood mononuclear cell (PBMC) samples obtained with only Ficoll-Hypaque gradient separation. The phenotype and light-scatter properties of these cells are indistinguishable from those of DC isolated by immunomagnetic sorting. For three colour flow-cytometric analysis of DCs, cells were labelled simultaneously with FITC-lin cocktail (CD3, CD11b, CD14, CD16, CD19, CD20, CD56), TRI-anti HLA-DR and PE-conjugated mAb recognising one of the following cell surface determinants CD1a, CD4, CD54, CD80, or CD86. The proportion of $lin^-HLA\text{-}DR^+$. The observations of this study suggest that the multidimensional flow-cytometric technique described might aid in identifying therapies that spare or boost immunity (see page 239). Claims 1-9, 22-27, at least, are considered to lack novelty and an inventive step in the light of this disclosure.

(continued)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of V. 2. Citations and explanations (Rule 70.7)

D7 discloses a method for enumerating the circulating $CD33^+$ (bright), $CD14^-$, $CD16^-$, $HLA\text{-}DR^+$ myeloid DC population by immunostaining and flow cytometry of lysed whole blood. An acquisition gate was established based on FSC and SCC that included both the lymphocyte and monocyte populations (mononuclear cells) but excluded most granulocytes and debris. In some experiments $CD33^+$ (bright), $CD14^-$, $CD16^-$, $HLA\text{-}DR^+$ cells were sorted using FACStarPLUS instrument. This document indicates that further studies are clearly warranted to establish normal ranges of circulating DC in large numbers of normal individuals and to examine the relationship between various pathological conditions and numbers of circulating DC of various subsets. Claims 1-2, 4-9, 16, 22 and 25, at least, are considered to lack novelty and an inventive step in the light of this disclosure.

D8 discloses the identification of five dendritic cell subsets in human tonsils based on HLA-DR, CD11c, CD13, and CD123 expression. A population of lin^- ($CD3^-$, $CD14^-$, $CD16^-$, $CD19^-$) DC-enriched cells were obtained by sorting on a flow cytometer. Triple labelling of sorted lin^- cells with CD11c, HLA-DR and CD123 confirmed that HLA-DRmod $CD11c^-$ DCs were a heterogeneous population based on CD123 expression. The phenotype of these subsets of DCs were further investigated. Table 2 discloses a tonsil DC subset that is HLA-DRmod, $CD11c^-$, $CD123^-$, $CD40^+$ and $CD45RA^{+/-}$ and $CD45RB^{+/-}$. Claims 1-2, 4-11, 16, 22-25 and 29, at least, are considered to lack novelty and an inventive step in the light of this disclosure.

D9 discloses a procedure for the simultaneous detection, quantitation and isolation of two distinct DC subsets in freshly isolated peripheral blood. The procedure is based on three- or four-colour flow cytometry. The threshold on forward scatter is adjusted to exclude debris. The two DC subsets identified are (i) $CD123^+$ (lin^- (CD3, CD14, CD16, CD19, CD20, CD56), $HLA\text{-}DR^{++}$, $CD123^{+++}$, $CD11c^-$) and (ii) $CD11c^+$ (lin^-, $HLA\text{-}DR^{+++}$, $CD123^+$ and $CD11c^{+++}$). Claims 1-2, 4-11, 16, 22-26, at least, are considered to lack novelty and an inventive step in the light of this disclosure.

D10 discloses alterations in the frequency of dendritic cell subsets in the peripheral circulation of patients with squamous cell carcinomas of the head and neck (SCCHN). Three-colour flow cytometry analysis was performed in which cells in the lymphocyte-monocyte light scatter gate were evaluated for the expression of lineage markers lin-FITC (CD3, CD14, CD16, CD19, CD20 and CD56) and DR-PerCP. Lin^- cells were evaluated for expression of CD11c and CD123 to identify $lin^-DR^+CD11c^+$ or $CD123^+$ DC subsets. This study found a striking and significant relative decrease in the percentage of $CD11c^+$ DC subsets in the peripheral circulation of SCCHN patients as compared to healthy individuals. This decrease was reproducibly seen in patients of both genders and of all ages. Decreased numbers of $CD11c^+$ DCs in the circulation of patients with SCCHN could be the consequence of cancer particularly because a recovery in the relative proportions of the CD11c+ subset was observed after tumour ablation (see page 1791 column 2). This document does suggest that $CD11c^+$ cells are derived from a less mature $lin^-DR+CD11c^-CD123^-$ precursor. Claims 1-11, 16, 22-26, at least, are considered to lack novelty and an inventive step in the light of this disclosure.

D11 discloses a method for obtaining, from human blood, DC characterised by a phenotype that is positive for HLA-DR and negative for CD3, CD14, CD16, CD19, CD20 and CD56, the method including fluorescently activated cell sorting (see claims 16-17). It is indicated that DC have been typically identified by their characteristic dendritic morphology and their cell surface phenotype characterised by a panel of mAbs against several markers such as CD1a, CD11b, CD11c, CD40, CD54, CD80, CD83, CD86 and CD123 (page 6 line 32-33). Figure 3A-D depicts the results of 3-colour FACS analysis of DC precursors following staining with PerCP-DR, FITC-lin and PE-CD86 antibodies. Claims 1-2, 4-12, 16, 17, 19, 22-27, at least, are considered to lack novelty and an inventive step in the light of this disclosure.

Industrial applicability (IA): Claims 1-29

Claims 1-29 are considered to possess industrial applicability in the field of biomedical testing.